A Study of Hypertension:
Participation of Central Adrenergic Mechanism on
the Angiotensin II-induced Hypertension

MAKOTO IIJIMA, M.D., NORIKATSU YAMAMOTO, M.D., YOSHIO KITAMURA, M.D.,
TADAO YASUGI, M.D., AND MICHI NOBU HATANO, M.D.

A variety of factors have been implicated in the pathogenesis of essential hypertension and the various predisposing factors and their interrelations have been extensively studied. While it is generally admitted as an established fact that hereditary predisposition constitutes a particularly important element among others, involvement of the sympathetic nervous system in the mechanisms of development and persistence of hypertension has been described. Although the precise mechanism remains obscure, the central pressor effect of intraventricularly administered angiotensin seems to be primarily caused by evoking a peripheral sympathetic discharge. And physiologic levels of brain norepinephrine might be necessary for the production of the angiotensin-induced pressor response following intraventricular administration. So interaction of angiotensin and the noradrenergic nerves in the brain may play an important role in raising blood pressure by the central angiotensin effect.

In our previous studies, intravenous administration of angiotensin reduced the rat hypothalamus norepinephrine with an accompanying decrease of MAO activity and an increase of COMT activity. However, intravenously administered angiotensin may influence limited areas of the brain because of the blood-brain barrier. So it will be necessary to administer angiotensin into the brain intraventricularly without obstruction by the blood-brain barrier and to determine the norepinephrine of the brain regions in order to investigate the interaction of angiotensin.

The present study was undertaken to investigate the involvement of the adrenergic mechanism in the central vasopressor effect angiotensin II through observation of changes of the rat hypothalamus and other brainstem norepinephrine by intravenous and intraventricular administration as well as of blood pressure changes in rabbits following injection with an α-adrenergic blocking agent (phentolamine) or with a β-adrenergic blocking agent (propranolol) into the lateral ventricle of the brain.

MATERIALS AND METHODS

I. Male Wistar rats, weighing from 400 to 500 g, and anesthetized with pentobarbital were used in the experiments. Angiotensin (Hypertensin-Ciba) was dissolved in distilled water, and the volume adjusted to 0.1% v/wt of rat for intravenous injection, or was dissolved in artificial cerebrospinal fluid (C.S.F. Merlis’ solution) for intraventricular perfusion.

After intravenous injection and/or intraventricular perfusion of angiotensin, the rats were killed. The brain tissue examined were separated into three portions. (A) hypothalamus,
(B) thalamus and midbrain, (C) pons and medulla oblongata according to Głowinski. The brain tissue was homogenized in 0.4N perchloric acid and the brain norepinephrine was extracted, separated through the cation exchange column and determined by the T.H.I. method modified by Weil-Malherbe. Angiotensin contained in C.S.F. was perfused intraventricularly (angiotensin 500 ng/min at a speed of 0.01 cc/min) for 20 minutes and the various doses of angiotensin (0.1, 0.25, 0.5 µg/kg) were injected intravenously, followed by further intraventricular perfusion with angiotensin for 15 minutes. The rats were killed at the end of the second perfusion and the brain norepinephrine contents were determined.

II. Male rabbits were anesthetized by intravenous injection with a solution of α-chloralose. The blood pressure was recorded on a polyocorder (Nippon Photoelectric) via a polyethylene tube passed into the femoral artery. The experiments were carried out to investigate the effects of α- and β-adrenergic blocking agents on the central pressor action of angiotensin, using injection into the ventricularis of angiotensin II, phenolamine 1000 µg/0.1 ml as an α-adrenergic blocker and dl-propranolol as a β-adrenergic blocking agent. Controls received comparable volumes (0.1 ml) of distilled water adjusted to the same pH. In all experiments, the various drugs were injected in volumes of 0.1 ml into the lateral ventricle.

RESULTS
I. (1) Effect of angiotensin (i.v.) on the rat hypothalamus and brainstem norepinephrine contents.

The norepinephrine contents of control rat hypothalamus and brainstem were 1.63 ± 0.02, 0.766 ± 0.01, 0.544 ± 0.008 µg/g for the hypothalamus, midbrain, and medulla oblongata respectively. On the other hand the contents were 1.52 ± 0.022, 0.734 ± 0.012, 0.544 ± 0.008 µg/g for the angiotensin group. The hypothalamus norepinephrine content of the angiotensin group was significantly less than that of the distilled water group (Fig. 1).

(2) Effect of angiotensin (i.v.) on the hypothalamus and brainstem norepinephrine contents under normal and angiotensin containing C.S.F. intraventricular perfusion.

Under normal C.S.F. intraventricular perfusion, norepinephrine contents of rat brain after intravenous administration of various doses of antitensin are shown in Table I. Significant alterations of the norepinephrine contents were obtained in the hypothalamus. Norepinephrine values were 1.64 ± 0.04, 1.52 ± 0.01, 1.53 ± 0.03, 1.48 ± 0.02 µg/g for the control, angiotensin 0.1, 0.25, 0.5 µg/kg respectively. Other parts of brainstem were not altered by the procedures.

Changes of the norepinephrine contents by intravenous administration of angiotensin to rats under intraventricular perfusion of C.S.F. containing angiotensin are shown in Table I. Generally, hypothalamus norepinephrine contents were more reduced by angiotensin perfusion than by normal C.S.F. perfusion. However there was a significant decrease of norepinephrine at a dose of 0.5 µg/kg compared with the distilled water group. Other brainstem norepinephrine contents were not affected by these procedures.

II. (1) Blood pressure changes following injection with angiotensin II into the lateral ventricle.

In response to injection with 0.2, 0.5, 1, or 3 µg antitensin into the lateral ventricle under α-chloralose anesthesia, rabbits each showed a trend to blood pressure elevation of which the average value, however, was not statistically significant. With a dose of 4–5 µg, as can be seen from Figure 2, the blood pressure began rising 1 minute after the injection and the elevation became significant at 3 minutes. The elevation was maximal 20 minutes after the injection, the mean blood pressure increase being 7.2 ± 1.5

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TABLE 1 THE EFFECT OF INTRAVENOUSLY (i.v.) ADMINISTERED ANGIOTENSIN ON THE NOREPINEPHRINE CONTENTS IN RAT HYPOTHALAMUS AND BRAINSTEM UNDER INTRAVENTRICULAR (i.v.t.) PERFUSION OF C.S.F. CONTAINING ANGIOTENSIN (µg/kg)

<table>
<thead>
<tr>
<th>Angiotensin (i.v.) µg/kg</th>
<th>Hypothalamus</th>
<th>Thalamus &amp; Midbrain</th>
<th>Pons &amp; Medulla Oblongata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal C.S.F. intraventricular perfusion.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (Control)</td>
<td>1.64 ± 0.04</td>
<td>0.744 ± 0.015</td>
<td>0.611 ± 0.016</td>
</tr>
<tr>
<td>0.1</td>
<td>1.52 ± 0.01**</td>
<td>0.736 ± 0.011</td>
<td>0.619 ± 0.013</td>
</tr>
<tr>
<td>0.25</td>
<td>1.53 ± 0.03*</td>
<td>0.722 ± 0.013</td>
<td>0.619 ± 0.031</td>
</tr>
<tr>
<td>0.5</td>
<td>1.48 ± 0.02***</td>
<td>0.721 ± 0.035</td>
<td>0.626 ± 0.025</td>
</tr>
</tbody>
</table>

Angiotensin C.S.F. intraventricular perfusion.

| 0 (Control)              | 1.54 ± 0.04  | 0.708 ± 0.033       | 0.600 ± 0.013            |
| 0.1                      | 1.48 ± 0.01  | 0.720 ± 0.016       | 0.595 ± 0.013            |
| 0.25                     | 1.40 ± 0.04  | 0.713 ± 0.050       | 0.575 ± 0.014            |
| 0.5                      | 1.39 ± 0.03* | 0.686 ± 0.042       | 0.592 ± 0.021            |

Values are means ± S.E. and are based on four to six determinations.
Differences from control are significant, *p < 0.05, **p < 0.02, ***p < 0.01.

Fig. 2. The effect of intraventricularly administered angiotensin on the rabbit mean arterial blood pressure.

(S.E.) mmHg.
(2) Central effect of α-adrenergic blocker (phenolamine) and angiotensin II.

No significant change in blood pressure occurred in 20 minutes after an intraventricular dose of 500 µg phenolamine alone, as compared with the control group. The blood pressure fell slightly by an average of −6.0 ± 1.7 (S.E.) mmHg
Fig. 3. The effect of intraventricularly administered phentolamine on the pressor response evoked by intraventricularly administered angiotensin in rabbits.

Significance: 10–15', P < 0.02; 20–30', P < 0.01.

Fig. 4. The effect of intraventricularly administered propranolol on the rabbit mean arterial blood pressure.

Significance: 15', P < 0.05; 20', P < 0.02; 30', P < 0.01.

at 30 minutes post injection. When 5 μg of angiotensin and 500 μg of phentolamine were administered simultaneously into the lateral ventricle, there was no pressor response and the suppression was significant at 10, 15 and 20 minutes after injection as compared with the blood pressure values observed after administration of 5 μg angiotensin alone (Fig. 3).

(3) Central effects of β-adrenergic blocker (propranolol) and angiotensin II

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In response to an intraventricular dose of 500 μg propranolol, the blood pressure of rabbits began to decline 1 minute after the injection and this decline lasted over the ensuing 3 hours (Fig. 4).

Animals receiving 5 μg of angiotensin and 100 μg of propranolol injected simultaneously into the lateral ventricle of the brain exhibited elevation of blood pressure which was somewhat greater than that observed in those given angiotensin alone (Fig. 5).

When 5 μg angiotensin was administered intraventricularly in combination with an increased dose of 500 μg propranolol, the blood pressure began increasing within 1 minute after the injection and showed a maximum increase by 41.7 ± 1.5 (S.E.) mmHg at 3–5 minutes. The blood pressure recovered from the hypertension 20 minutes after the injection and, thereafter, tended to decline. The vasopressor response was significantly greater than that seen following administration of 5 μg angiotensin alone (Fig. 5).

Rabbits injected with 50 μg propranolol in 0.05 ml and 10 minutes later with 5 μg angiotensin in 0.05 ml, both intraventricularly, dis-
played blood pressure elevation of which the pattern was generally similar to that observed after administration of 5 µg angiotensin alone. The elevation of blood pressure, nevertheless, was significantly greater at 15 and 20 minutes, i.e. 10.8 ± 0.12 (S.E.) mmHg and 12.4 ± 1.0 (S.E.) mmHg respectively, as compared with that produced by administration of 5 µg angiotensin alone.

When propranolol at various concentrations was injected intravenously in combination with angiotensin, there was a tendency for the blood pressure elevation to be slightly suppressed unlike the response to central administration although the degree of elevation was practically comparable to that seen after administration of angiotensin alone.

**DISCUSSION**

It is generally accepted that angiotensin interacts with the peripheral sympathetic nerve endings. Palaic and Khairallah suggested that angiotensin inhibits the norepinephrine re-uptake at the membrane of the nerve cell. These interactions between angiotensin and the norepinephrine terminals may occur in the brain. The hypothalamus, which is innervated by ascending noradrenergic fiber originating in the lower brainstem, is rich in norepinephrine terminals. So perfusion of the third ventricle with angiotensin may release norepinephrine, or inhibit the norepinephrine re-uptake at norepinephrine terminals in the hypothalamus, and the accumulated norepinephrine may be washed out or metabolized by COMT at an extracellular space. In the present study, hypothalamic norepinephrine was reduced by intravenicularly perfused angiotensin but other parts of brainstem were not affected. This result may be interpreted well by the interaction described above.

With respect to the decrease of norepinephrine by the intravenous injection of angiotensin, two possible interpretations will be considerable, namely the angiotensin directly influenced the hypothalamus norepinephrine through the defect of the blood brain barrier or the peripheral response to the angiotensin indirectly affected the hypothalamus to reduce its norepinephrine content. In the present study hypothalamic norepinephrine was reduced more by intravenously administered angiotensin than by intraventriculally perfused angiotensin. (Fig. 6). Considering that the decrease by the perfusion with a relativel high concentration of

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angiotensin for 35 minutes was less than by intravenous administration it is likely that the peripheral response to angiotensin indirectly effected the hypothalamus norepinephrine. Namely another mechanism, which could be excited by the peripheral response to the angiotensin, may participate in the decrease of norepinephrine in hypothalamus. This second response to the peripheral action of angiotensin might imply participation of a baroreceptor reflex. Palaic and Khairallah\textsuperscript{27} showed that electric stimulation of the central end of right vagal nerve of rat produced a significant release of norepinephrine and decrease of acid metabolites into the cerebro-ventricular space that was potentiated by intraventricular perfusion of angiotensin.

Results of the study suggest that the intraventricularly perfused angiotensin reduces the norepinephrine from "one area" and the intravenous administration of angiotensin reduces that from "another area" of hypothalamus. Fig.6 illustrates decreases of hypothalamic norepinephrine by the intraventricular and intravenous administration of angiotensin. The degree of decrease by the intraventricular administration seems to be constant with or without intravenous administration of angiotensin. This is compatible with the suggestion mentioned above. So two noradrenergic functional sites might be considered in hypothalamus, one is related to the pressor response of the central angiotensin effect and the other to the baroreceptor reflex mechanism. And hypothalamus may participate in the regulation of blood pressure with its noradrenergic nervous activity.

In the rabbit experiments administration of an $\alpha$-blocker (phenolamine) into the lateral ventricle of the brain produced no significant change in blood pressure in rabbits. When angiotensin and phenolamine were injected simultaneously into the cerebral ventricle there was a significant suppression of the pressor response to angiotensin. Similar results obtained by experiments in rats were reported by Severs et al.\textsuperscript{29} That the central hypertensive response to angiotensin is inhibited by phenolamine suggests possible involvement of the $\alpha$-adrenergic receptor in the central pressor effect of angiotensin. Conversely, lowering of blood pressure with bradycardia developed immediately following administration of 500 $\mu$g dl-propranolol into to cerebral ventricle. Simultaneous administration of angiotensin and propranolol brought about a remarkably greater increase in blood pressure than that produced by injection with angiotensin alone (Fig. 7). From the fact that the elevation of blood pressure observed following peripheral administration of angiotensin alone in a dose corresponding to one-tenth that of intraventricular injection practically did not differ from that produced by simultaneous peripheral injection of angiotensin and propranolol, it would be deducible that no significant alteration in chemical properties of angiotensin takes place.

As for influence of $\beta$-adrenergic blocking agents central pressor effect of angiotensin, Severs et al\textsuperscript{29} described that the central pressor effect of angiotensin was inhibited in unanesthetized rats by propranolol. However, reports of such findings as yet are few, and the remarkable blood pressure elevation in out experiment of anesthetized rabbits has not yet be reported. The initial pressor response immediately after the administration of propranolol and then followed by a fall of blood pressure was reported in dogs\textsuperscript{30} This initial reaction did not occur in adrenalectomized dogs. Other reports\textsuperscript{31,33} suggested the local anesthetic effect or stimulant effect of $\beta$-blocking agents. Even based on these reports, the enhanced pressor effect in our experiment of simultaneously administered angiotensin and propranolol is extremely difficult to interpret.

**SUMMARY**

I. Interactions of angiotensin II and norepinephrine in the rat hypothalamus and other brainstem were studied by intraventricular perfusion or/intravenous administration.

(1) Intravenous administration of angiotensin reduced the hypothalamus norepinephrine content.

(2) Intraventricularly perfused angiotensin reduced the hypothalamus norepinephrine but did not alter that in other parts of brainstem. Hypothalamus norepinephrine was reduced more by intravenous administration of angiotensin than by intraventricularly perfused angiotensin.

II. The involvements of adrenergic mechanism in the central pressor effect of angiotensin II were studied through observation of blood pressure changes following injection of angiotensin II, phenolamine and propranolol into the rabbit lateral ventricle of rabbit brain.

(1) Intraventricular administration of phenolamine suppressed the central mediated pressor
respons of angiotensin.

(2) Simultaneous intraventricular administration of propranolol enhanced the angiotensin pressor effect in anesthetized rabbits.

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